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Apolipoprotein L1 gene variants associate with hypertension-attributed nephropathy and the rate of kidney function decline in African Americans

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Despite intensive antihypertensive therapy there was a high incidence of renal end points in participants of the African American Study of Kidney Disease and Hypertension (AASK) cohort. To better understand this, coding variants in the *apolipoprotein L1* (*APOL1*) and the *nonmuscle myosin heavy chain 9* (*MYH9*) genes were evaluated for an association with hypertension-attributed nephropathy and clinical outcomes in a case–control study. Clinical data and DNA were available for 675 AASK participant cases and 618 African American non-nephropathy control individuals. *APOL1* G1 and G2, and *MYH9* E1 variants along with 44 ancestry informative markers, were genotyped with allele frequency differences between cases and controls analyzed by logistic regression multivariable models adjusting for ancestry, age, and gender. In recessive models, *APOL1* risk variants were significantly associated with kidney disease in all cases compared to controls with an odds ratio of 2.57. In AASK cases with more advanced disease, such as a baseline urine protein to creatinine ratio over 0.6 g/g or a serum creatinine over 3 mg/dl during follow-up, the association was strengthened with odds ratios of 6.29 and 4.61, respectively. *APOL1* risk variants were consistently associated with renal disease progression across medication classes and blood pressure targets. Thus, kidney disease in AASK participants was strongly associated with *APOL1* renal risk variants.

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African Americans are more likely to develop progressive kidney disease compared with European Americans,[1] and this risk extends across the leading causes of end-stage kidney disease (ESKD): diabetes, hypertension-attributed kidney disease, and glomerulonephritis.[2] In particular, for hypertension-attributed nephropathy, there is a threefold increase in incidence of ESKD for African Americans overall, which is magnified at younger ages.

The genetic contribution to the increased prevalence of hypertension-attributed ESKD is now better understood. In the past several years, a region on chromosome (chr) 22

associated with renal disease in African Americans was identified using mapping by admixture disequilibrium. This region contains the *nonmuscle myosin heavy chain 9* (*MYH9*) and *apolipoprotein L1* (*APOL1*) genes. Initial studies that detected the association in this region showed a very strong association of *MYH9* with human immunodeficiency virus–associated nephropathy and focal segmental glomerulosclerosis (FSGS),[3,4] as well as an association with nondiabetic forms of ESKD.[3,5]

More recent studies have shown that there is an even stronger association of the *APOL1* gene with FSGS and hypertension-attributed ESKD than with *MYH9* among African Americans. Controlling for *APOL1* risk alleles showed no residual effect of the *MYH9* gene, whereas controlling for *MYH9* genotype did not significantly weaken the effect of *APOL1*.[6,7] Although these data suggested little role for the *MYH9* risk alleles, it has subsequently been shown that *MYH9* risk alleles are associated with increased risk of nondiabetic chronic kidney disease (CKD) and diabetic ESKD in individuals of European ancestry.[8,9] In these studies, there was no risk attributable to *APOL1* genotypes; however, the frequency of the *APOL1* risk alleles was extremely low among European Americans.

To further understand the role of A*POL1* and *MYH9* genetic variants in hypertension-attributed CKD, we have performed a case–control study and evaluated the rate of decline of measured iothalamate glomerular filtration rate (GFR). African American cases were patients with CKD from the African American Study of Kidney Disease and Hypertension (AASK) who at baseline had subnephrotic proteinuria or no proteinuria, measured iothalamate GFR of <65 ml/min per 1.73 m^2, and clinically diagnosed hypertensive nephropathy.[10,11] Cases were compared with African American controls with normal kidney function as determined by history and serum creatinine measurement, some with mild or moderate hypertension.

RESULTS

The characteristics of the African American cases and African American control subjects are presented in Table 1. All of the cases had hypertension and CKD. Of the controls, 410 were questioned about the presence of hypertension, and of these 41.7% (171) reportedly were hypertensive based on physician report or the use of antihypertensive medications. Those with CKD were predominantly male and older compared with controls and had similar body mass index and population admixture. Cases had a low baseline GFR and elevated serum creatinine, whereas controls had a mean serum creatinine concentration of 1.0 mg/dl.

All reported *APOL1* and *MYH9* SNPs were in Hardy–Weinberg equilibrium (HWE) in the AASK participants and in the controls. Although analyses were performed

Table 1 | Demographic and clinical information (% or mean (median) ± s.d.)

	Cases *N*=675	Controls *N*=618	*P*-value
Sex (% female)	40%	57%	<0.0001
Age (years)	54.1 (55) ± 10.6	49.1 (48) ± 11.7	<0.0001
BMI (kg/m^2)	31.0 (30) ± 6.6	30.1 (29) ± 7.1	0.0103
Serum creatinine (mg/dl)	1.99 (1.8) ± 0.7	1.00 (0.9) ± 0.26	<0.0001
Mean baseline GFR (ml/min per 1.73 m^2)	47.2 (49) ± 13.4	NA	—
Hypertension (% yes)	100%	41.7% of patients who were surveyed	<0.0001
YRI admixture (%)	0.89 (0.93) ± 0.11	0.89 (0.92) ± 0.10	0.34

Abbreviations: BMI, body mass index; GFR, glomerular filtration rate; NA, not available; YRI, Yoruban.
African American cases were from the AASK (African American Study of Kidney Disease and Hypertension) study; 618 African American controls were recruited at Wake Forest School of Medicine, and 171 of 410 evaluated reported hypertension.

Table 2 | Chromosome 22 genotype results

Gene	SNP	C22 position	Risk allele	Risk allele fraction, cases	Risk allele fraction, controls	*P*-value, recessive model	OR, recessive model	95% CI
APOL1	rs16996616	36661891	A	0.09	0.07	3.18E-01	2.1	(0.49, 9.04)
*APOL1*G1	rs73885319	36661906	G	0.28	0.21	2.97E-06	3.47	(2.06, 5.85)
*APOL1*G1	rs60910145	36662034	G	0.28	0.20	2.77E-06	3.54	(2.09, 6.00)
*APOL1*G2	rs71785313	36662051	C	0.16	0.13	1.46E-01	1.72	(0.83, 3.57)
APOL1G1G2				0.44	0.34	1.39E-08	2.57	(1.85, 3.55)
MYH9	rs11912763	36684722	A	0.24	0.19	1.36E-03	2.56	(1.44, 4.54)
MYH9 E1	rs2032487	36695428	C	0.69	0.61	2.11E-04	1.56	(1.23, 1.97)
MYH9 E1	rs4821481	36695942	C	0.70	0.60	3.41E-05	1.65	(1.30, 2.09)
MYH9	rs5750250	36708483	A	0.42	0.50	1.07E-03	0.62	(0.47, 0.83)
MYH9 EI	rs3752462	36710183	T	0.76	0.73	1.81E-02	1.33	(1.05, 1.68)

Abbreviations: OR, odds ratio; SNP, single nucleotide polymorphism.
Genotype results are shown for chromosome 22 alleles, including those in *APOL1*, those comprising the G1 and G2 alleles, and in *MYH9* (including those SNPs comprising the E1 haplotype). Data were adjusted for age, sex, and population admixture. Admixture outliers were excluded from analysis, leaving 675 cases and 618 controls.

for the additive, dominant, and recessive models, the largest effect sizes (odds ratios (ORs)) were observed for the recessive model. This is consistent with the results of other studies.[6,7] The tables show the results for only the recessive model; associations for the additive and dominant models are presented in Supplementary Table S1 online, and *APOL1* genotype frequencies are presented in Supplementary Table S2 online. The results of the primary single-SNP analysis are shown in Table 2. The associations of *APOL1* G1 SNPs with CKD were highly significant and associated with approximately a 3.5-fold risk for CKD for G1. The G2 SNP was not significant, but, as in other studies[6,7] was present at lower allele frequencies than the other SNPs. It should be noted that the more frequent G1 allele tends to mask the effect of G2, as the two variants occur on opposing chromosomes.

Table 3 | Case vs. control comparisons, adjusted for *MYH9* SNP rs4821481 or *APOL1* combined risk alleles

Adjusted for *MYH9* SNP rs4821481	*APOL1* SNP	*P*-value, cases/ controls	Odds ratio	95% CI
	rs16996616	2.44E-01	2.49	(0.54, 11.55)
	rs73885319 (G1)	1.87E-04	2.78	(1.62, 4.76)
	rs60910145 (G1)	1.69E-04	2.83	(1.65, 4.88)
	rs71785313 (G2)	2.85E-01	1.50	(0.72, 3.14)
Adjusted for *APOL1* combined risk alleles	***MYH9* SNP**			
	rs11912763	4.08E-01	1.32	(0.69, 2.52)
	rs2032487 (E1)	1.20E-01	1.23	(0.95, 1.59)
	rs4821481 (E1)	4.67E-02	1.30	(1.00, 1.69)
	rs5750250	6.92E-02	0.76	(0.56, 1.02)
	rs3752462 (E1)	6.73E-01	1.06	(0.82, 1.36)

Abbreviations: CI, confidence interval; SNP, single nucleotide polymorphism.
The top half of the table shows the effect of *APOL1* SNPs, adjusted for the *MYH9* SNP rs48212481 using a recessive model. The bottom half of the table shows the effect of *MYH9* SNPs, adjusted for *APOL1* combined risk alleles. Data were adjusted for age, sex, body mass index, and population admixture.

MYH9 SNPs showed significant associations with CKD, but weaker than the *APOL1* G1 and combined G1–G2 association. Analyses were repeated controlling for either the combined *APOL1* G1–G2 risk alleles or the *MYH9* E1 SNP rs4821481 using the recessive model, and the data are shown in Table 3. Controlling for the *APOL1* risk alleles attenuated the effect of *MYH9* SNPs: the E1-tagging SNP rs4821481 remained of borderline statistical significance. Controlling for rs4821481 did not markedly decrease the effect of the *APOL1* G1 alleles.

Association results for the combined risk of *APOL1* G1-G2 alleles on CKD are presented in Table 4 and the *MYH9 E1* haplotype in Table 5. The statistical significance and OR increased when comparing patients with more advanced renal disease as measured by serum creatinine concentration and proteinuria. This was particularly significant for the *APOL1* risk alleles, but was also apparent for the *MYH9* E1 risk haplotype. The sensitivity analysis comparing subsets of controls phenotyped for hypertension and normal blood pressure (BP) shows ORs similar to that of the controls as a

Table 4 | Logistic regression model of the effect of *APOL1* risk alleles on clinical phenotype

Outcome	*N* (cases)	*N* (controls)	*P*-value	OR, recessive model	95% CI
All AASK cases vs. controls	663	579	1.39E-08	2.57	(1.85, 3.55)
Cases with serum creatinine >2 mg/dl or ESKD vs. controls	330	579	1.99E-12	3.64	(2.54, 5.21)
Cases with serum creatinine >3 mg/dl or ESKD vs. controls	216	579	5.60E-15	4.61	(3.14, 6.76)
Cases with urine PCR <0.22 g/g vs. controls	457	579	0.0219	1.55	(1.07, 2.26)
Cases with urine PCR >0.22 g/g vs. controls	204	579	2.70E-15	4.85	(3.28, 7.18)
Cases with urine PCR >0.60 g/g vs. controls	105	579	2.62E-14	6.29	(3.92, 10.11)
Hypertensive kidney disease vs. hypertensive controls	663	158	0.0013	2.40	(1.41, 4.08)
Hypertensive kidney disease vs. non-hypertensive controls	663	220	8.52E-07	3.62	(2.17, 6.05)

Abbreviations: AASK, African American Study of Kidney Disease and Hypertension; CI, confidence interval; ESKD, end-stage kidney disease; OR, odds ratio; PCR, protein/creatinine ratio.
Analysis using *APOL1* risk alleles (G1 and G2) was adjusted for age, sex, and admixture.

Table 5 | Logistic regression analysis for the effect of the *MYH9* E1 haplotype on clinical outcomes

Outcome	*N* (cases)	*N* (controls)	*P*-value	OR, recessive model	95% CI
Case–control	622	592	0.0002	1.60	(1.25, 2.05)
Serum creatinine >2 mg/dl or ESKD	311	592	5.99E-06	1.99	(1.48, 2.68)
Serum creatinine >3 mg/dl or ESKD	201	592	1.05E-08	2.68	(1.91, 3.76)
Urine PCR <0.22 g/g	431	592	0.0461	1.33	(1.01, 1.76)
Urine PCR >0.22 g/g	189	592	1.26E-05	2.38	(1.68, 3.36)
Hypertensive controls	622	167	0.0619	1.42	(0.98, 2.06)
Non-hypertensive controls	622	227	0.0019	1.74	(1.23, 2.47)

Abbreviations: CI, confidence interval; ESKD, end-stage kidney disease; OR, odds ratio; PCR, protein/creatinine ratio.
Shown are the results of logistic regression analysis using a recessive model for the outcomes among cases compared with controls, adjusted for age, sex, and population admixture.

Table 6 | Effects of *APOL1*, blood pressure target, and medication class on rate of decline of GFR in the AASK Trial

		Slope of iothalamate GFR			
Medication class	BP arm	*APOL1* non-risk	*APOL1* risk	*P*-value	Heterogeneity *P*-value
ACE inhibitor	Low	−1.47 ± 0.22	−2.68 ± 0.40	0.0202	
	Usual	−1.50 ± 0.23	−2.84 ± 0.41	0.0023	
β-Blocker	Low	−1.59 ± 0.24	−2.22 ± 0.41	0.3776	
	Usual	−2.01 ± 0.24	−2.70 ± 0.40	0.1736	
Calcium channel blocker	Low	−2.05 ± 0.34	−2.72 ± 0.60	0.2542	
	Usual	−2.18 ± 0.33	−3.17 ± 0.62	0.2050	
Meta-analysis				4.29E-05	0.6257

Abbreviations: AASK, African American Study of Kidney Disease and Hypertension; ACE, angiotensin-converting enzyme; *APOL1* non-risk, less than two (G1+G2) risk variants; *APOL1* risk, two (G1+G2) risk variants; BP, blood pressure; GFR, glomerular filtration rate.
Based on least-squares projected slope of iothalamate GFR in AASK Trial phase, beginning 6 months after enrollment, *APOL1* genotypes were significantly associated with rate of kidney function decline, in contrast to no effect of blood pressure treatment goal or medication class. The meta-analysis compares *APOL1* risk and non-risk genotypes combining all six treatment cells.

whole; the differences in *P*-values and ORs when using these as control subsets, compared with using combined controls, may be due to the differences in sample size.

Results of analyses testing *APOL1* genotype effects on renal disease progression, accounting for BP medication and goal BP, are reported in Table 6. This analysis demonstrated that *APOL1* risk (recessive) predicted the rate of progression of renal disease; the nonsignificant heterogeneity *P*-value reveals that the association between *APOL1* groups and progression did not differ significantly across medication class and BP treatment arm.

Analysis of the achieved BP at 1 year post randomization in these 675 AASK participants did not reveal significantly different systolic BP, diastolic BP, or mean BP in cases randomized to either low or usual BP treatment arm based on the presence of two vs. less than two *APOL1* risk variants. In the case of diastolic BP, the usual treatment arm had a trend toward a lower mean ± s.d. diastolic BP in the *APOL1* two risk–variant group (84.2 ± 1.29 mm Hg) vs. the *APOL1* less than two risk–variant group (86.2 ± 0.76 mm Hg; $P = 0.0565$). Heterogeneity *P*-values comparing usual and low BP treatment arms on the basis of *APOL1* risk and nonrisk were nonsignificant, revealing that the effect of the *APOL1* risk variants on BP did not differ by treatment arm (heterogeneity $P = 0.6240$ systolic BP; $P = 0.3721$ diastolic BP; $P = 0.4447$ mean BP).

To test whether the AASK Trial inference changed after adjusting for the strong effect of the *APOL1* risk variants, we computed a general linear model with age, gender, and *APOL1* G1/G2 risk variants under a recessive model as covariates. We tested the effects of the BP arm adjusting for the medication class and the above covariates. We also tested for the effects of the medication class, adjusting for the BP arm and the above covariates. Specifically, there was no effect of the BP arm adjusting for age, gender, and *APOL1* G1/G2 risk variants under a recessive model and medication class group ($P = 0.19$). There was no effect of the medication class group, adjusting for age, gender, and *APOL1* G1/G2 risk variants under a recessive model and the BP arm ($P = 0.13$). Finally, there was no effect of the combined medication treatment group and the BP arm, adjusting for age, gender, and *APOL1* G1/G2 risk variants under a recessive model ($P = 0.23$). There was no evidence that the magnitude of the effect of the *APOL1* variants differed as a function of the BP arm ($P = 0.56$) or medication class ($P = 0.18$).

DISCUSSION

The results presented here demonstrate that nephropathy risk variants in the *APOL1* gene, and to a lesser extent *MYH9*, are significantly associated with CKD attributed to essential hypertension in nondiabetic AASK participants compared with controls. Evidence of genetic association was most robust in individuals with progressive renal functional decline and higher baseline levels of proteinuria. It is unlikely that these variants are associated with essential hypertension *per se*, as the results were consistent when comparing hypertensive AASK cases with controls with or without high BP. These results strongly suggest that progressive kidney disease attributed to hypertensive nephrosclerosis in AASK participants, particularly in those with higher baseline levels of proteinuria, lie in the spectrum of FSGS-related kidney disease, as in idiopathic FSGS, as well as human immunodeficiency virus–related, C1q-related, and idiopathic collapsing forms of FSGS. Focal global glomerulosclerosis, arteriolar nephrosclerosis, and interstitial scarring are commonly present in the renal biopsies of AASK participants[12] and appear to reside in this disease spectrum based on *APOL1* association.

Subjects in the AASK trial were randomized to angiotensin-converting enzyme (ACE) inhibitor, dihydropyridine calcium channel blocker, or β-blockade with low and usual BP targets (e.g., a 3 × 2 factorial design). Subsequently, all participants in the continuation study, the AASK Cohort Study, received ACE inhibitors with a low BP goal. Despite this aggressive therapy, 54% of AASK patients experienced the primary outcome (doubling of creatinine, ESKD, or death).[13] These analyses demonstrate that *APOL1* risk (recessive model) predicted the least-squares projected slope of iothalamate GFR during the AASK Trial phase. The nonsignificant heterogeneity *P*-value reveals that medication class and BP treatment arm did not significantly differ in effect on progression of kidney disease across groups, accounting for *APOL1*-associated genetic risk. This is consistent with the AASK Trial results, demonstrating that a lower BP goal did

not affect renal disease progression. However, ACE inhibitors did slow the progression of renal disease in AASK relative to β-blockers and calcium channel blockers; the inability to detect this in the current study may be because of the smaller sample size. These results suggest that the failure of intensive treatment of BP to halt the progression of renal disease is associated with the role of *APOL1* gene variants. New clinical targets are urgently required to combat this severe genetic form of kidney disease.

It is unclear why ACE inhibition, which often benefits patients with heavy proteinuria, had less of a protective effect in AASK. In this subset of AASK participants, ACE inhibition (regardless of usual or low BP treatment arm) did not significantly impact renal disease progression after accounting for *APOL1*. Not all AASK Trial or AASK Cohort participants were included in these genetic analyses, and hence low power may contribute. In addition, AASK excluded participants with >2.5 g of proteinuria per day at baseline; most participants had a far lower level of proteinuria. Therefore, the protective effect of ACE inhibition might have been reduced owing to the generally lower levels of proteinuria.

Important clinical and histological differences exist between African Americans and European Americans in the kidney disease that is labeled hypertensive nephrosclerosis. African Americans develop ESKD attributed to high BP earlier in life than European Americans.[2] In addition, successful treatment of hypertension more effectively slows nephropathy progression in European Americans, relative to African Americans.[14,15] Despite being labeled with the same clinical diagnosis (hypertensive nephrosclerosis), African Americans exhibit greater degrees of solidified glomerulosclerosis and arteriolonephrosclerosis, whereas European Americans have greater degrees of obsolescent, collapsed glomeruli.[12,16] These racial differences have long hinted at different causative factors for renal disease progression. *APOL1* nephropathy risk variants are present at high frequency in African-derived populations and are virtually absent in European-derived and Asian populations. Therefore, the clinical differences in nondiabetic renal disease may primarily relate to variation in *APOL1*, as it has been associated with shorter survivals of African American–donated kidneys in the setting of deceased donor transplantation.[17] Importantly, kidneys from African American donors without *APOL1* risk variants demonstrated excellent allograft survival, similar to European American–donated kidneys. Thus, *APOL1* genotypes, not race, convey risk for nephropathy.

Aggressive treatment of high BP in AASK failed to significantly slow nephropathy progression in nondiabetic African Americans with hypertension and CKD, particularly among those who lacked proteinuria at baseline;[11] the use of ACE inhibitors slowed progression compared with β-blocker or calcium channel blocker,[18] but long-term progression rates on ACE inhibitors remain high.[13] Genetic influences that may not be sensitive to ACE inhibitors or BP control have previously been shown to contribute to this propensity for progressive loss of kidney function among AASK participants, including variants in the adrenergic pathway[19] and the homocysteine pathway.[20] We now show that in this region of chr 22 genetic variation in *APOL1*, and to a lesser extent *MYH9*, is strongly associated with progression of kidney disease and may offer a new perspective on hypertension-attributed renal disease. *MYH9* has effects independent of *APOL1* on nephropathy risk in those with sickle cell disease[21] and in individuals of European ancestry.[8,9] There are now abundant data that current therapies have limited ability to slow progression of kidney disease. It is therefore critical to understand the function of *APOL1* in order to develop improved therapeutic options to slow progression of nondiabetic kidney disease.

MATERIALS AND METHODS

Subjects

The AASK clinical trial compared the effects of three different drugs and two BP targets on progression of hypertension-attributed nephropathy in 1094 African Americans recruited at 21 clinical sites in the United States. Study subjects were self-identified African Americans between the age of 18 and 70 years, who had a diastolic BP >95 mm Hg and a measured iothalamate GFR between 20 and 65 ml/min per 1.73 m^2. Selected exclusion criteria included evidence of a clinical cause other than hypertension for kidney disease, diabetes mellitus or fasting blood glucose level >140 mg/dl, urine protein/creatinine ratio (PCR) >2.5 g/g, secondary hypertension, or accelerated or malignant hypertension in the preceding 6 months.

Control subjects were recruited at the Wake Forest School of Medicine and consisted of 948 African American subjects with serum creatinine concentrations <1.5 mg/dl in men or <1.3 mg/dl in women, of whom 410 were questioned about the presence of hypertension and 171 (41.7%) reported having high BP.

Ethics approvals

The AASK clinical protocol was approved by the Institutional Review Board (IRB) of each participating institution, and each patient provided informed consent. After the trial was underway, subjects were approached to contribute DNA as part of an Ancillary Study that was approved by the IRB at each participating study site; 850 subjects consented and contributed blood for DNA extraction. Control participants provided DNA and clinical information through a protocol approved by the Wake Forest School of Medicine IRB. All controls provided written informed consent.

Genetic analysis

DNA was genotyped for 10 SNPs on chr 22 using TaqMan assays (Applied Biosystems, Foster City, CA), including the four *MYH9* E1 haplotype SNPs (rs4821480, rs2032487, rs4821481, rs3752462) and two other SNPs in the *MYH9* region (rs5750250 and rs11912763), the *APOL1* G1 nonsynonymous SNPs (rs73885319, rs60910145), the *APOL1* G2 6-bp insertion/deletion (rs71785313), and the nonsynonymous SNP rs16996616 in the *APOL1* gene. An additional 44 ancestry informative marker SNPs were genotyped in cases and controls. The ancestry informative markers included chr 1: rs1334336; rs2365669; rs9725312; chr 2: rs11890727; rs1868929; chr 3: rs7627605; chr 4: rs2725267; rs12503758; rs2545258; chr 5: rs7727623; rs6881896; rs7712675; chr 6: rs4946888; rs9388989; rs686406; chr 7: rs3823831; rs10488004; rs520556; chr 8: rs4732942;

rs13439780; chr 9: rs449090; rs7873820; chr 10: rs1733742; rs570677; chr 11: rs647756; rs7952397; chr 12: rs7963493; rs4767461; chr 13: 1572018; rs11148886; chr 14: rs1956424; rs12897140; chr 15: rs4451923; chr 16: rs10775349; rs7198976; chr 17: rs8074370; chr 19: rs901792; rs2231738; chr 20: rs1418032; rs6046024; rs1028546; chr 21: rs220245; chr 22: rs12159761; and chr 23: rs6520141. Admixture analysis was performed with ADMIXMAP (http://homepages.ed.ac.uk/pmckeigu/admixmap/index.html), using allele frequencies from CEU (Utah Residents with Northern and Western European Ancestry) and Yoruban (YRI) HapMap samples. Genotype data for CEU and YRI were included in the analysis as anchoring populations. Samples with YRI proportion <0.4 (three cases) were excluded from the analysis. After quality control for missing phenotypic data, duplicates, genotyping failures, and admixture outliers, 675 cases and 618 controls were available for this study. Results for SNP rs4821480 were not included in the analysis because of technical difficulties with the TaqMan assay; its proxy, rs4821481, was retained in the analysis. All reported SNPs conformed to Hardy–Weinberg expectations for genotype frequencies. Slightly different cell counts relate to covariate availability across analyses.

Analyses were performed to examine the combined risk of *APOL* G1–G2 alleles and the *MYH9* E1 haplotype for different clinical phenotypes. These analyses were performed in subgroups of AASK cases who had or developed severe CKD during the study, defined as follows: (1) developing ESKD or serum creatinine >2 mg/ml; or (2) developing ESKD or serum creatinine >3 mg/dl. Stratification by proteinuria was evaluated as in prior AASK analyses, which demonstrated that patients with urine PCR $\geqslant 0.22$ g/g have a greater risk of progression to ESKD than those with less proteinuria.[11] Sensitivity analyses were performed comparing cases with controls who had reported hypertension or normal BP.

Analyses testing *APOL1* genotype effects on renal disease progression, accounting for BP medication and goal BP, were performed on AASK participants. Renal disease progression was based on the least-squares projected slope of iothalamate GFR during the AASK Trial phase (beginning at 6 months to avoid acute medication effects). A test for whether the association varied by BP target and/or medication class (heterogeneity *P*-value), as well as the six individual tests of the association within the BP target-medication class combinations, was performed.

Quality control

The accuracy of the E1 *MYH9* and *APOL1* TaqMan assays was validated by resequencing a group of DNAs from 60 African American donors and by genotyping several hundred African and European controls, all of which conformed to HWE expectations and had allele frequencies similar to those reported in dbSNP (http://www.ncbi.nlm.nih.gov/snp). In this study, we included 10% duplicate samples within and between plates to control for genotyping errors; there was 100% concordance for all SNPs. All but one SNP, rs4824180, were in HWE and showed expected patterns of linkage disequilibrium. We therefore elected to regenotype all *MYH9* E1 SNPs and the *APOL1* SNPs to confirm genotype assignment; there was a 100% match for all SNP genotypes. SNP rs4824180 was removed from the analysis both because it was out of HWE ($P=0.0001$) and because the SNP showed an implausible pattern of linkage disequilibrium with neighboring SNPs.

Data analysis

Single SNP analysis was performed using logistic regression via SNPGWA (http://www.phs.wfubmc.edu/public/bios/gene/downloads.cfm), adjusting for age, gender, and admixture. The primary analysis compared all the cases with all the controls. Subgroup analyses were performed stratifying the cases by degree of renal failure: serum creatinine (>2 mg/dl or ESKD vs. controls or serum creatinine >3 mg/dl or ESKD vs. controls) and degree of proteinuria (urine PCR $\geqslant 0.22$ g/g vs. controls and urine PCR <0.22 g/g vs. controls). As not all the controls were phenotyped for self-reported hypertension, sensitivity analyses were performed comparing cases with controls with documented hypertension or with documented normotension.

To determine whether both *MYH9* and *APOL1* risk alleles were associated with CKD, we performed additional single SNP analyses adjusting for *APOL1* G1–G2 combined allele risk (defined as being homozygous for the G1 allele (rs73885319 allele G) or homozygous for the G2 allele (rs71785313 deletion), or heterozygous for both G1 and G2 alleles (one copy of the risk 'allele' for each marker), to assess for residual risk of the *MYH9* SNPs.

The role of the *MYH9* risk haplotype (recessive model for E1 risk haplotype, consisting of SNPs rs2032487, rs4821481, rs3752462) was analyzed using logistic regression controlling for age, gender, admixture, and G1–G2 alleles combined risk.

DISCLOSURE

National Institutes of Health has applied for a patent on the clinical use of MYH9 genetic testing for kidney disease susceptibility, with coinventors CAW, GWN, and JBK. All remaining authors declare no conflict of interest.

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SUPPLEMENTARY MATERIAL

Table S1. AASK Case vs. Control Age, gender, and admixture adjusted 675 cases, 618 controls.

Table S2. Frequencies of APOL1 genotypes.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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